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Article

Clinical Utility of Germline Genetic Testing in Japanese Men Undergoing Prostate Biopsy

Shusuke Akamatsu, MD, PhD,[1,2,*,‡] Naoki Terada, MD, PhD,[3,‡] Ryo Takata, MD, PhD,[2,4] Hidefumi Kinoshita, MD, PhD,[5] Kimihiro Shimatani, MD,[6] Yukihide Momozawa, PhD,[7] Michio Yamamoto, PhD,[8,9,10] Harue Tada, PhD,[8] Naoki Kawamorita, MD, PhD,,[11] Shintaro Narita, MD, PhD,[12] Takuma Kato, MD, PhD,[13] Masahiro Nitta, MD, PhD,[14] Shuya Kandori, MD, PhD,[15] Yusuke Koike, MD,[16] Johji Inazawa, MD, PhD,[17] Takahiro Kimura, MD, PhD,[16] Hiroko Kimura, MD,[1] Takahiro Kojima, MD, PhD,[15] Toshiro Terachi, MD, PhD,[15] Mikio Sugimoto, MD, PhD,[13] Tomonori Habuchi, MD, PhD,[12] Yoichi Arai, MD, PhD,[11] Shingo Yamamoto, MD, PhD,[6] Tadashi Matsuda, MD, PhD,[5] Wataru Obara, MD, PhD,[4] Toshiyuki Kamoto, MD, PhD,[3] Takahiro Inoue, MD, PhD,[18] Hidewaki Nakagawa, MD, PhD,[3,#] Osamu Ogawa, MD, PhD,[1,#]; on behalf of the PCSSNP study group

[1]Department of Urology, Kyoto University Graduate School of Medicine, Kyoto, Japan, [2]RIKEN Center for Integrative Medical Sciences, Laboratory for Cancer Genomics, Kanagawa, Japan, [3]Department of Urology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan, [4]Department of Urology, School of Medicine, Iwate Medical University, Iwate, Japan, [5]Department of Urology and Andrology, Kansai Medical University, Osaka, Japan, [6]Department of Urology, Hyogo College of Medicine, Hyogo, Japan, [7]Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan, [8]Institute for Advancement of Clinical and Translational Science (iACT), Kyoto University Hospital, Kyoto, Japan, [9]Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan, [10]RIKEN Center for Advanced Intelligence Project, Kanagawa, Japan, [11]Department of Urology, Tohoku University Graduate School of Medicine, Miyagi, Japan, [12]Department of Urology, Akita University Graduate School of Medicine, Akita, Japan, [13]Department of Urology, Faculty of Medicine, Kagawa University, Kagawa, Japan, [14]Department of Urology, Tokai University School of Medicine, Kanagawa, Japan, [15]Department of Urology, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan, [16]Department of Urology, Jikei University School of Medicine, Tokyo, Japan, [17]Department of Molecular Cytogenetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan and [18]Department of Nephro-Urologic Surgery and Andrology, Mie University Graduate School of Medicine, Mie, Japan

[‡]Contributed equally as first authors.
[#]Contributed equally as senior authors.
***Correspondence to:** Shusuke Akamatsu, MD, PhD, Department of Urology, Kyoto University Graduate School of Medicine, 54 Shougoinkawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan (e-mail: akamats@kuhp.kyoto-u.ac.jp).

Abstract

Background: Multiple common variants and also rare variants in monogenic risk genes such as *BRCA2* and *HOXB13* have been reported to be associated with risk of prostate cancer (PCa); however, the clinical setting in which germline genetic testing could be used for PCa diagnosis remains obscure. Herein, we tested the clinical utility of a 16 common variant–based polygenic risk score (PRS) that has been developed previously for Japanese men and also evaluated the frequency of PCa-associated rare variants in a prospective cohort of Japanese men undergoing prostate biopsy. **Methods:** A total of 1336 patients undergoing first prostate biopsy were included. PRS was calculated based on the genotype of 16 common variants, and sequencing of 8 prostate cancer–associated genes was performed by multiplex polymerase chain reaction based target sequencing. PRS was combined with clinical factors in logistic regression models to assess whether addition of PRS improves the prediction of biopsy positivity. **Results:** The top PRS decile was associated with an odds ratio of 4.10 (95% confidence interval = 2.46 to 6.86) with reference to the patients at average risk, and the estimated lifetime absolute risk approached 20%. Among the patients with prostate specific antigen 2-10 ng/mL who had prebiopsy magnetic resonance imaging, high PRS had an equivalent impact on biopsy positivity as a positive magnetic resonance imaging finding. Rare variants were detected in 19 (2.37%) and 7 (1.31%) patients with positive and negative biopsies, respectively, with *BRCA2* variants being the most prevalent. There was no association between PRS and high-risk rare variants. **Conclusions:** Germline genetic testing could be clinically useful in both pre- and post-PSA screening settings.

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Genetic risk factors are among the most well-known causes of prostate cancer (PCa). Genome-wide association studies (GWAS) have identified 269 single-nucleotide polymorphisms (SNPs) associated with PCa (1-6), signifying the contribution of common variants in PCa development. In addition, mutations in genes, such as *BRCA1* and 2 and *ATM*, account for up to 10%-20% of metastatic PCa cases (7,8), and rare variants in *HOXB13* are also known to be associated with the risk of developing PCa (9-11).

Currently, PCa is commonly diagnosed using prostate-specific antigen (PSA) testing followed by prostate needle biopsy. Because the diagnostic accuracy of a single PSA test is low, especially in the gray zone (4-10 ng/mL), additional parameters such as PSA density, PSA velocity, and multiparametric magnetic resonance imaging (MRI) are also considered when selecting patients to undergo biopsy. Family history of PCa is one of the clinical parameters that prompts PSA screening at a younger age or earlier biopsy in patients with gray-zone PSA. However, family history information is often incomplete or imprecise. The polygenic risk score (PRS) based on common variants is a method that explicitly captures the common polygenic components of family history (12). It is estimated that the common variants identified to date account for 40% of PCa heritability (1). Thus, PRS is expected to be clinically useful in identifying patients who are at a high risk of developing PCa.

Although the potential utility of germline genetic testing including PRS (13,14) and target sequencing of select monogenic genes (8) has been reported for PCa, the clinical setting in which these tests would be useful is not well defined. In the present study, we prospectively tested the utility of a 16-SNP–based PRS we have previously developed and validated (13), and we also performed target sequencing of 8 well-known monogenic risk genes reported to be associated with PCa development in patients undergoing prostate needle biopsy.

Methods

The key experimental methods are described below. Further details can be found in the Supplementary Methods (available online).

Study Design

The Prostate Cancer Susceptibility Single Nucleotide Polymorphism (PCSSNP) study is a multi-institutional prospective study to evaluate the utility of PRS in prostate cancer diagnosis. A total of 1394 patients were registered, and after excluding ineligible patients, 1336 were included in the final analysis (Supplementary Figure 1, available online). The primary endpoint of the study was to assess whether the addition of PRS to clinical parameters would improve the prediction of biopsy positivity in men undergoing prostate needle biopsy. In addition, the present study aimed to examine the prevalence of germline mutations in known monogenic PCa-associated genes among Japanese patients undergoing prostate needle biopsy. Together, the study was designed to assess the clinical utility of germline genetic testing for prostate cancer diagnosis. The study was approved by the ethical committees at each institution and was registered at the University Hospital Medical Information Network Clinical Trials Registry (15) (UMIN000019278).

Genotyping of Common Variants and PRS Calculation

A multi-index polymerase chain reaction (PCR)-based target sequencing method was used to sequence the target regions, as previously described (16). We have previously created a genetic risk prediction model using 16 SNPs that were confirmed to be associated with PCa in a Japanese cohort (13). The model was created using 689 PCa patients and 749 healthy individuals and validated using 2 independent sets of cohorts comprising 3249 PCa patients and 6281 healthy individuals. Logistic regression analysis was conducted to create the model. Odds were estimated for each sample using the following formula,

$$odds = \frac{p(x)}{1-p(x)} = \exp\left(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots\right),$$

where β_n are the regression coefficients of each SNP, x_n is the number of risk alleles at each SNP locus, and $p(x)$ is the probability of developing PCa conditional on the number of risk alleles x_n. The β_n values calculated for each SNP in our previous study (13) were applied. For the current study, each sample was scored for each of the 16 SNPs with the number of risk alleles, and the odds calculated from the formula were used as PRS. The result of the case-control analysis for the 16 SNPs using the current cohort is presented in Supplementary Table 1 (available online).

Target Genome Sequencing and Annotation of Variants

For target genome sequencing, we selected 8 genes (*ATM*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *HOXB13*, *NBN*, and *PALB2*) for which we have conducted a similar analysis previously in a large cohort of unselected Japanese PCa patients and healthy controls (9). In the previous study, we chose the 8 genes based on a review article describing that rare variants in these genes show high penetrance for PCa (17). We analyzed the complete coding regions and 2-base pair flanking intronic sequences of the 8 genes by multiplex PCR-based target sequencing as described previously (9). We assigned clinical significance (pathogenic, benign, or uncertain) for all variants of the 8 genes. First, we determined the clinical significance based on the pathogenicity assertions registered in ClinVar (18). The variants reported as "pathogenic" or "likely pathogenic" were considered pathogenic. Additionally, novel variants not registered in ClinVar that were predicted to lead to a loss-of-function event or deletions that disrupt the coding sequences were also considered pathogenic. We used the same procedure for all genes except *HOXB13*. For *HOXB13*, p.Gly84Glu, and p.Gly135Glu variants have been reported to be pathogenic (10,11), whereas we previously reported p.Gly132Glu and p.Gly17Val variants as novel pathogenic variants (9); thus, they were considered pathogenic in this study.

Statistical Analysis

A logistic regression model was created for each combination of clinical factors and PRS, and their performance in predicting biopsy positivity was compared using receiver operating characteristic analysis. For multivariable analysis, only the parameters that were statistically significantly associated ($P < .05$) with outcomes in the univariate analysis were included in the models. For PSA density (PSAD) dichotomy, a cutoff of 0.2 ng/mL/mL was applied.

To compare variables between groups, categorical and continuous data were analyzed using Pearson χ^2 test and student *t*

Table 1. Patient characteristics and the results of final biopsy[a]

Characteristic	PCa(+)	PCa(−)	Total
Total No. of patients (%)	801 (60.0)	535 (40.0)	1336 (100)
Age, y			
Mean (SD)	70.6 (7.2)	66.0 (8.2)	68.7 (7.9)
Median (min, max)	71.0 (47, 90)	67.0 (37, 91)	69.0 (37, 91)
Digital rectal exam, No. (%)			
PCa suspected	279 (34.9)	44 (8.2)	323 (24.2)
PCa not suspected	520 (65.1)	490 (91.8)	1010 (75.8)
N/A	2	1	3
PSA, ng/mL			
Mean (SD)	80.3 (575.5)	7.4 (4.7)	51.1 (446.9)
Median (min, max)	8.7 (0.03, 14 426)	6 (0.7, 42.7)	7.3 (0.03, 14 426)
Prostate volume, mL^3			
Mean (SD)	32.0 (17.8)	41.3 (20.0)	35.8 (19.2)
Median (min, max)	28 (7.3, 175)	37.8 (5.7, 186)	30.1 (5.7, 186)
PSA density, ng/mL/mL			
Mean (SD)	1.99 (11.55)	0.21 (0.16)	1.28 (8.98)
Median (min, max)	0.34 (0.002, 265.7)	0.17 (0.03, 1.64)	0.43 (0.002, 265.7)
Family history, No. (%)			
Yes	43 (5.6)	39 (7.8)	82 (6.5)
No	720 (94.3)	464 (92.2)	1184 (93.5)
N/A	38	32	70
Suspicion of PCa on MRI, No. (%)			
Yes	402 (87.2)	164 (57.3)	566 (75.8)
No	59 (12.8)	122 (42.7)	181 (24.2)
N/A	340	249	589
No. of biopsy cores			
Median (min, max)	12 (2,20)	12 (8, 22)	12 (2,22)

[a]max = maximum; min = minimum; MRI = magnetic resonance imaging; N/A = not available; PCa = prostate cancer; PSA = prostate-specific antigen.

test or Wilcoxon rank sum test, respectively. The association of each SNP was assessed using an additive model, and the Cochran-Armitage trend test was used to investigate the association between each SNP and PCa.

All analyses were performed using R statistical software (version.3.5.3) or JMP Pro v.14 (SAS Institute, Cary, NC, USA). All statistical tests were 2-sided with a statistical significance level of 0.05.

Results

Patient and Tumor Characteristics

An outline of the patient cohort is shown in Supplementary Figure 1 (available online). At the first biopsy, 778 patients were diagnosed with PCa (Supplementary Table 2, available online). Of the 558 patients not diagnosed with PCa at initial biopsy, during the median follow-up of 30.5 months (interquartile range = 15-38) after biopsy, 72 were rebiopsied at the discretion of the attending physician, and 23 were diagnosed with PCa. At the final data collection, 801 patients were diagnosed by biopsy, and 535 were not diagnosed with PCa (Table 1). Of the 889 patients with PSA levels 2-10 ng/mL, 446 were diagnosed with PCa and 443 had no PCa detected (Supplementary Table 3, available online). Because prebiopsy MRI was not mandated, MRI was performed in only 747 patients of the entire cohort, and 468 of those patients had PSA 2-10 ng/mL. Age, abnormal rectal exam results, PSA levels, prostate volume, and MRI findings were all statistically significantly associated with PCa in both the entire cohort and among patients with PSA levels 2-10 ng/mL. The characteristics of the detected PCa are summarized in Table 2. A total of 674 patients had localized disease (N0, M0), and 62 (7.7%) patients had nonclinically significant cancer (Gleason score [GS] $3+3=6$, $\leq$ T2a, positive biopsy core ≤ 2, and total positive core length ≤ 6 mm). A total of 409 (51.1%) patients had high-risk or advanced cancer (GS ≥ 8 or $\geq$ T3a or PSA ≥ 20 or N+, or M+).

Performance of PRS in Combination With Clinical Parameters to Predict PCa

The median PRS for the entire cohort, using the logistic regression model, was 0.91, ranging from 0.09 to 7.58. When patients were grouped by PRS categories, the estimated odds ratio (OR) for men in the top 10% of the PRS (90%-100% PRS category) was 4.10 (95% confidence interval [CI] = 2.46 to 6.86) compared with men with an average risk in the 40%-60% PRS category after adjusting for age at biopsy (Table 3; Supplementary Table 4, available online). The odds ratio for men in the top 1% of the PRS distribution (99%-100%) was 5.37 (95% CI = 1.13 to 25.55). The area under the curve (AUC) of the PRS for positive biopsy results was 0.630 (95% CI = 0.600 to 0.660) for the entire cohort (Supplementary Figure 2, available online). There was no statistically significant difference in PRS between those with clinically significant and nonclinically significant PCa ($P=.32$) or between high-risk and nonhigh-risk PCa ($P=.58$) (Supplementary Figure 3, available online). Next, we focused on patients with PSA levels 2-10 ng/mL, in whom the performance of PSA to predict PCa is poor. The AUC of the PRS was 0.618 (95% CI = 0.580 to 0.654). The AUC of PSA alone was 0.575 (95% CI =

Table 2. Characteristics of diagnosed tumor

Tumor characteristics	No. (%)
Gleason score[a]	
3 + 3 = 6	149 (18.6)
3 + 4 = 7	171 (21.3)
3 + 5 = 8	4 (0.5)
4 + 3 = 7	142 (17.7)
4 + 4 = 8	179 (22.3)
4 + 5 = 9	102(12.7)
5 + 3 = 8	1 (0.1)
5 + 4 = 9	45 (5.6)
5 + 5 = 10	8 (1.0)
cT stage	
T1c	282 (35.2)
T2a	243 (30.3)
T2b	57 (7.1)
T2c	68 (8.5)
T3a	83 (10.4)
T3b	44 (5.5)
T4	17 (2.1)
Tx	7 (0.9)
cN stage	
N0	709 (88.5)
N1	79 (9.9)
Nx	13 (1.6)
cM stage	
M0	704 (87.9)
M1	79 (9.9)
Mx	18 (2.2)
Nonclinically significant cancer[b]	62 (7.7)
Clinically significant cancer	739 (92.3)
Intermediate/low-risk cancer	392 (48.9)
High-risk cancer[c]	409 (51.1)

[a]The highest Gleason score is shown for the patients who have had multiple biopsies after initial active surveillance.
[b]Nonclinically significant cancer: fulfill all of the right (Gleason score 3 + 3 = 6, ≤T2a, positive biopsy core ≤2, total positive core length ≤6 mm).
[c]High-risk cancer: fulfill 1 of the right (Gleason score ≥8, ≥T3a, prostate-specific antigen ≥20, N+, M+).

Table 3. Odds ratio by polygenic risk score (PRS) category

PRS category, %	OR[a] (95% CI)
0-10	0.75 (0.49 to 1.17)
10-20	0.96 (0.61 to 1.49)
20-30	1.04 (0.67 to 1.61)
30-40	0.92 (0.60 to 1.43)
40-60	1.00 (referent)
60-70	1.39 (0.90 to 2.17)
70-80	2.04 (1.29 to 3.23)
80-90	3.27 (1.98 to 5.37)
90-100	4.10 (2.46 to 6.86)
99-100	5.37 (1.13 to 25.55)

[a]Odds ratio (OR) is calculated by logistic regression analysis with presence of PCa as the objective variable and PRS category and age as explanatory variables. CI = confidence interval.

0.537 to 0.612); however, the AUC of PSAD statistically significantly increased to 0.719 (95% CI = 0.685 to 0.751; $P < .001$) (Figure 1). When PRS and PSAD were combined in a logistic regression model, the AUC statistically significantly increased to 0.747 (95% CI = 0.713 to 0.777; $P = .002$). A multivariable analysis incorporating age, PSAD, digital rectal exam (DRE), and PRS showed that these parameters were all independently associated with biopsy positivity (Supplementary Table 5, available online). The AUC including all the variables was 0.784 (95% CI = 0.753 to 0.813), and 0.756 (95% CI = 0.723 to 0.786) in a model without age. Because the decision to rebiopsy was at the physician's discretion in the present study, we also conducted a sensitivity analysis based solely on the results of the first biopsy (Supplementary Figure 4, available online). The AUC including all the variables was 0.779 (95% CI = 0.747 to 0.807).

To further test the performance of PRS, we also evaluated the models without PSA (Supplementary Figure 5, available online). The AUC of age alone was 0.627 (95% CI = 0.590 to 0.663) and increased to 0.679 (95% CI = 0.643 to 0.713) with the addition of PRS. The AUC approached 0.702 (95% CI = 0.667 to 0.735) when DRE was also incorporated. However, the addition of PSA to this model did not improve the discriminative performance (AUC = 0.709, 95% CI = 0.675 to 0.742). We also evaluated whether the number of biopsy cores affected the performance of the model; however, the increase in AUC was minimal (AUC = 0.711, 95% CI = 0.677 to 0.744).

Among the 468 patients with PSA 2-10 ng/mL who had MRI data available, MRI was also independently associated with a positive biopsy (Table 4; Supplementary Table 6, available online). The overall effect size of PRS on biopsy positivity was comparable to that of MRI after adjusting for age, PSAD, and DRE. When PRS was included in a multivariable model as a dichotomous variable with a cutoff of 2.0, the odds ratio of PRS of 2.0 or more was 2.88 (95% CI = 1.50 to 5.53), which was comparable to that of positive MRI (OR = 2.78, 95% CI =1.70 to 4.55). The AUC was 0.742 (95% CI = 0.696 to 0.784) with the model incorporating clinical parameters (PSAD and DRE) alone, increased to 0.770 (95% CI = 0.725 to 0.809) with the addition of PRS, and was the highest when MRI was also included (AUC = 0.795, 95% CI = 0.751 to 0.832). The increase in AUC with the addition of PRS and MRI was statistically significant ($P = .01$ and $P = .02$, respectively). Taken together, even though PSAD is the strongest predictor of positive biopsy in patients with PSA 2-10 ng/mL, PRS and MRI moderately improve the prediction of biopsy positivity.

Utility of PRS to Identify Patients Who May Benefit From Early Screening

Taking advantage of the large discovery cohort from GWAS and the PCSSNP cohort, we next explored whether PRS could be used to identify patients who are at high risk for developing PCa and may benefit from early PSA screening. To this end, we set an arbitrary cutoff of PRS at 3.0, 2.5, and 2.0 to define patients at high risk for PCa. In our Japanese GWAS cohort used for the PRS creation and validation (13), which comprised 3983 PCa patients and 7030 healthy individuals, 3.4%, 5.4%, and 9.7% of cases were classified as high risk based on PRS cutoffs of 3.0, 2.5, and 2.0, respectively (Supplementary Table 7, available online). In the PCSSNP cohort, 3.6%, 6.2%, and 13.1% of patients were classified as high risk. At all cutoffs, high genetic risk was associated with PCa diagnosis with odds ratio higher than 3.0 (Table 5). In the PCSSNP cohort, genetically high-risk patients were diagnosed earlier than nonrisk patients based on all cutoffs (Supplementary Table 8, available online). As an alternative way to investigate the impact of PRS, we calculated the absolute risk for a given age for each PRS category based on the age-specific prostate cancer incidence and age-specific mortality

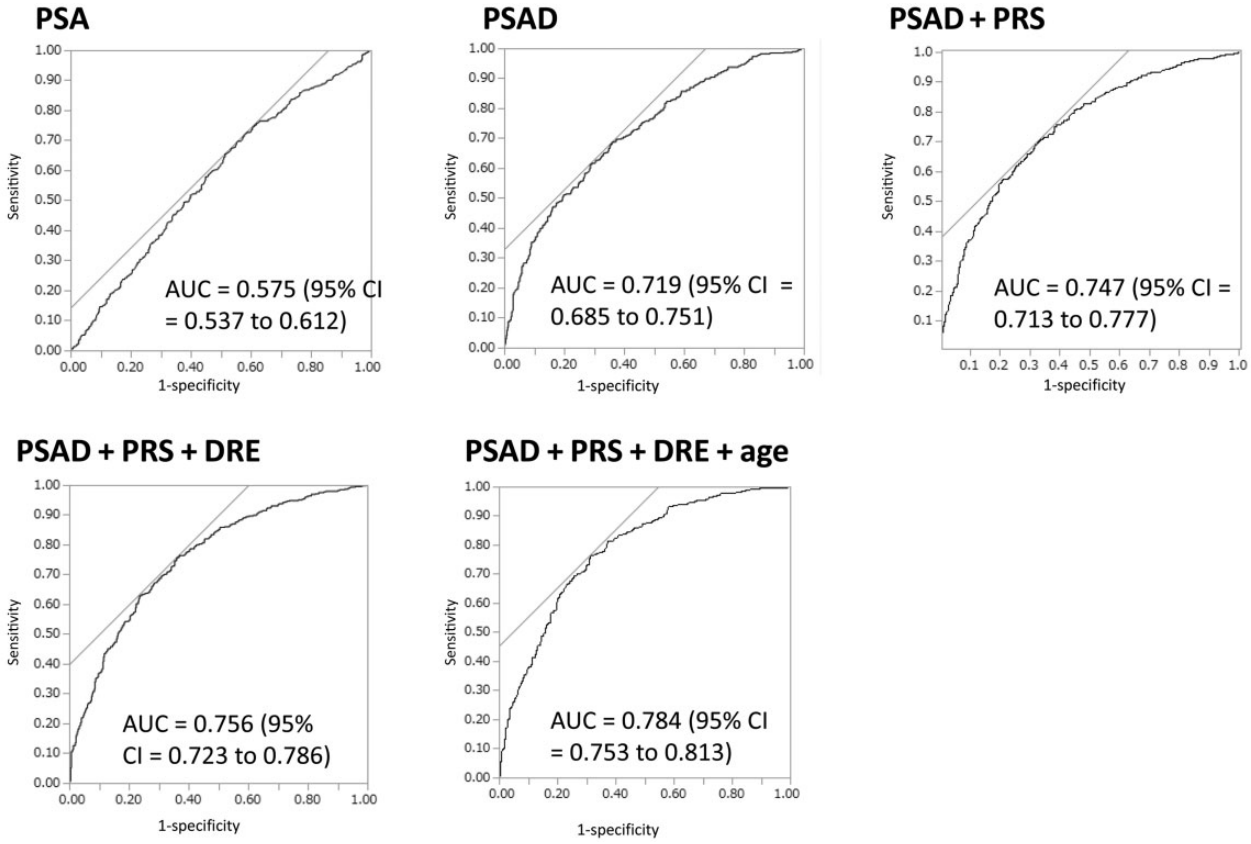

Figure 1. Diagnostic performance of logistic regression models incorporating clinical parameters and PRS. ROC analysis was performed for each combination of clinical parameters and PRS, and AUC (95% confidence intervals [CIs]) were calculated. AUC = area under the curve; DRE = digital rectal exam; PSA = prostate-specific antigen; PSAD = prostate-specific antigen density; PRS = polygenic risk score; ROC = receiver operating characteristics.

Table 4. Logistic regression analysis incorporating MRI, PSAD, age, PRS, and DRE

Parameter	OR[a] (95% CI)	P[b]
PSAD > 0.2 (≤0.2 as reference)	3.89 (2.54 to 5.95)	<.001
MRI positive (negative as reference)	2.78 (1.70 to 4.55)	<.001
PRS ≥ 2.0 (<2.0 as reference)	2.88 (1.50 to 5.53)	.001
DRE positive (negative as reference)	2.08 (1.71 to 3.70)	.01

[a]Odds ratio (OR) is calculated by logistic regression analysis with presence of prostate cancer as the objective variable and PSAD, MRI, PRS, and DRE findings and age as explanatory variables. CI = confidence interval; DRE = digital rectal exam; MRI = magnetic resonance imaging; PRS = polygenic risk score; PSAD = prostate-specific antigen density.
[b]Two-sided Pearson χ^2 test.

rates of Japanese individuals (Supplementary Figure 6, available online). Absolute risk increased with genetic risk and approached 20% in men in the top decile. We also examined the association between PRS and family history. Mean PRS of patients with a family history of PCa was 1.39 (standard deviation (SD) = 0.09) and was statistically significantly higher than in those without it (1.12 [SD = 0.02]; P = .001), suggesting a strong positive association between PRS and family history. Collectively, these data indicate that PRS is a robust index of hereditary components of PCa, and defining genetically high-risk patients based on PRS could be complementary to family history in clinical settings.

Monogenic Rare Variants in Japanese Undergoing Prostate Biopsy

Overall, 26 (1.95%) patients harbored a pathogenic mutation in 1 of the 8 genes (Table 6; Supplementary Table 9, available online). Pathogenic variants were identified in 19 (2.37%) and 7 (1.31%) patients with positive and negative biopsies, respectively. The most frequently mutated gene was *BRCA2*, followed by *HOXB13* and *ATM*. Two of the 3 PCa patients with *ATM* mutations and 4 of the 6 PCa patients with *BRCA2* mutations had high-risk PCa. In contrast, although 5 of the 6 patients with a *HOXB13* mutation had PCa, only 1 of them had high-risk PCa, suggesting that mutations in this gene may not be associated with disease aggressiveness. Similarly to a previous large-scale Japanese case-control study, 5 of the 6 mutations in *HOXB13* were p.Gly132Glu (46805561 C>T), which has been reported as a subpopulation-specific variant in Japanese (9). All patients with this mutation were positive for PCa in the present study. There were no patients with p.Gly84Glu and p.Gly135Glu mutations, which have been reported in European and Chinese populations (10,11). PRS among patients with monogenic risk variants was evenly distributed from 0.33 to 3.50, suggesting that genetic high risk defined by PRS and monogenic risk variants is completely independent.

Discussion

In the present study, odds ratio of the top 10% of the PRS (90%-100% category) was 4.10 (95% CI = 2.46 to 6.86). This was

Table 5. Odds ratio of genomically high-risk patients determined by PRS at different cutoffs[a]

PRS cutoff	PCSSNP cohort Age-adjusted OR (95% CI)[b]	Model creation cohort Age-adjusted OR (95% CI)[b]
PRS ≥3 vs PRS <3	6.86 (2.65 to 17.77)	3.65 (2.93 to 4.57)
PRS ≥2.5 vs PRS <2.5	4.93 (2.60 to 9.37)	3.79 (3.17 to 4.52)
PRS ≥2.0 vs PRS <2.0	3.10 (2.09 to 4.62)	3.55 (3.11 to 4.06)

[a]Patients were dichotomized into "high" and "nonhigh" risk at arbitrary PRS cutoffs. CI = confidence interval; OR = odds ratio; PCSSNP = Prostate Cancer Susceptibility Single-Nucleotide Polymorphism; PRS = polygenic risk score.
[b]Odds ratio of high-risk patients with reference to nonhigh-risk patients were calculated by logistic regression analysis.

comparable to that reported for Asians using PRS constructed from all 269 reported PCa-associated SNPs (OR = 4.15, 95% CI = 3.33 to 5.17) (1) and supports the high discriminative performance of our ancestry-specific PRS. To date, the clinical setting in which PRS established from common variants can be used remains obscure. We showed that in patients preselected to undergo prostate needle biopsy based on elevated PSA or other clinical factors, the addition of PRS to clinical parameters, such as PSAD, moderately improved the prediction of biopsy positivity. Notably, for patients with gray-zone PSA and genetic high risk, defined as PRS of 2 or more, accounting for approximately 10% of patients, PRS had an equivalent impact on biopsy positivity to a positive MRI finding. A positive MRI is a strong factor that prompts prostate biopsy in patients with gray-zone PSA, and the present data suggest that PRS could be equally important in deciding who to biopsy.

We also evaluated the potential role of PRS in patients who had not been screened for PCa. Recently, it has been reported that men in the top 10% risk by PRS have almost 40% estimated lifetime risk of developing PCa among Caucasians and 25% among Asians (1). We showed that the estimated lifetime risk of developing PCa for men in the top decile is almost 20%; however, this number may be underestimated by insufficient implementation of PSA screening in Japan compared with Western countries. Nonetheless, our data confirmed PRS use in defining patients at high risk for developing PCa. The present study also showed that although only 6.5% of patients had a positive family history of PCa, PRS was statistically significantly higher in patients with a family history of PCa. With an overall low incidence of PCa among Japanese patients, the proportion of patients with a positive family history is small. In addition, family history is sometimes not accurately remembered and liable to recall bias. PRS combined with sequencing of monogenic risk variants may be as useful as family history in identifying those who may benefit from earlier PSA screening.

The prevalence of rare variants in the 8 PCa-associated genes in a large Japanese cohort has been reported previously (9). Similar to that study, the overall prevalence of rare variants was 1.95% and higher in patients with a positive biopsy. However, the prevalence in biopsy-negative patients was 1.31%, which was higher than that observed in 12 366 (0.8%) healthy controls in a previous study. This indicates that compared with healthy individuals, patients undergoing prostate needle biopsy may be genetically closer to patients diagnosed with PCa, even when biopsy results are negative. The most frequently altered gene was *BRCA2*, followed by *HOXB13* and *ATM*. For *HOXB13*, we exclusively detected p.Gly132Glu and p.Gly17Val variants, and all 5 patients with p.Gly132Glu variants were biopsy positive, suggesting a strong association between this variant and PCa in Japanese populations. Interestingly, among those with *HOXB13* variants, only 1 had high-risk PCa. One patient (PCS0963) was diagnosed with metastatic PCa at the age of 51 years. The patient had a *CHEK2* p.R519* pathogenic variant, which is located in the coding region of the nuclear localization signal of *CHEK2* (19). This variant has been observed in patients with breast, ovarian, prostate, and uterine cancers (20). Detection of a rare variant in a gene known to be associated with PCa other than *BRCA1* and 2, *HOXB13*, and *ATM* in a young patient presenting with metastatic PCa suggests that, although rare, there are patients who would benefit from genetic testing of genes other than the more commonly altered genes.

A strength of the present study is that we have examined and validated the clinical utility of a PRS that has been established and validated in completely independent Japanese cohorts, which can avoid overfitting the PRS. Furthermore, prostate biopsy was performed in all patients, and biopsy-negative patients were followed for a few years to check for undetected cancers at the initial biopsy. Similarly to other PRS reported previously, our PRS could not discriminate between clinically significant and nonclinically significant cancer or high-risk and nonhigh-risk cancers. However, only 7.7% of PCa detected was nonclinically significant cancers, and more than half was high-risk PCa, suggesting that combined with other clinical parameters, the risk of overdiagnosis is low even when PRS is introduced into clinical practice.

The present study had several limitations. First, the rate of biopsy positivity was relatively high, because many patients were carefully selected to undergo biopsy. To confirm the generalizability of the present data, we also calculated the age-adjusted odds ratio in the cohort we used to validate the same PRS in our previous study (13) comprised of unselected PCa patients and healthy controls (Supplementary Table 9, available online). The age-adjusted odds ratio for men in the top PRS decile was 3.22 (95% CI = 2.73 to 3.81), which was lower than that in the PCSSNP cohort; however, the 95% confidence interval overlapped. Second, the decision to biopsy was at the physician's discretion. Although possible selection bias may affect the generalizability of the data presented, the AUC of PRS was similar to those reported previously (1,13). In addition, for patients with PSA 2-10 ng/mL who had MRI data available, most of the patients were initially screened by PSA and underwent MRI before the decision to biopsy, which is the current standard practice in Japan. Therefore, the data is generalizable to contemporary patients who are screened similarly. Third, the decision to rebiopsy was at the physician's discretion and possibly created selection bias. Therefore, we conducted a sensitivity analysis based on the results of the first biopsy, which showed that the impact of rebiopsy on the performance of the model was minimal.

In summary, we have shown that combined with clinical parameters, a PRS could be used to decide whether prostate biopsy will be recommended in men already screened for PSA and

Table 6. Clinical characteristics of the patients with monogenic rare variants in the 8 PCa-associated genes[a]

Patient ID	Gene	Variant	Protein alteration	Cancer/ noncancer	Age, y	PSA	Family history	Gleason score	cT stage	cN stage	cM stage	PRS	High-risk prostate cancer
PCSSNP0509	*ATM*	11_108124544_AC_A	p.His635fs	Noncancer	64	4.9	No	—	—	—	—	2.32	—
PCSSNP1071	*ATM*	11_108201089_C_T	p.Arg2486*	Cancer	71	14.5	Yes	4 + 4 = 8	T1c	N0	M0	0.38	Yes
PCSSNP1186	*ATM*	11_108164048_C_T	p.Asp1540Asp	Cancer	75	5.29	No	5 + 4 = 9	T2b	N0	M0	2.35	Yes
PCSSNP0355	*ATM*	11_108155202_T_C	ATM: splice donor intron variant	Cancer	74	7.24	No	3 + 4 = 7	T2a	N0	M0	0.72	No
	BRIP1	17_59878769_G_A	BRIP1: p. Gln329*										
PCSSNP1276	*BRCA2*	13_32906888_GA_G	p.Asp427fs	Noncancer	60	4.58	Yes	—	—	—	—	0.68	—
PCSSNP1040	*BRCA2*	13_32972674_G_T	p.Glu3342*	Noncancer	37	4.656	No	—	—	—	—	1.03	—
PCSSNP0371	*BRCA2*	13_32914065_CAATT_C	p.Ile1859fs	Noncancer	67	6.46	No	—	—	—	—	0.55	—
PCSSNP0498	*BRCA2*	13_32914065_CAATT_C	p.Ile1859fs	Cancer	73	7.91	No	3 + 3 = 6	T2a	N0	M0	0.38	No
PCSSNP0522	*BRCA2*	13_32920978_C_T	p.Arg2318*	Cancer	78	12.81	No	4 + 4 = 8	T3a	N0	M0	3.50	Yes
PCSSNP0402	*BRCA2*	13_32914893_ATAACT_A	p.Asn2135fs	Cancer	83	8.9	No	4 + 5 = 9	T1c	N0	M0	1.54	Yes
PCSSNP0806	*BRCA2*	13_32913763_T_A	p.Tyr1757*	Cancer	64	5.66	No	3 + 4 = 7	T2a	N0	M0	0.78	No
PCSSNP1306	*BRCA2*	13_32906627_GC_G	p.Arg414*	Cancer	81	181.8	No	4 + 4 = 8	T3b	N0	M0	0.81	Yes
PCSSNP0491	*BRCA2*	13_32907420_GA_G	p.Ile605fs	Cancer	72	6.16	No	3 + 4 = 7	T2a	N0	M0	1.87	No
PCSSNP1185	*BRCA2*	13_32912698_T_TA	p.Thr1403fs	Cancer	59	176	No	5 + 4 = 9	T3b	N0	M0	0.69	Yes
PCSSNP0713	*BRIP1*	17_59770807_TG_T	p.Pro853fs	Noncancer	83	25.7	No	—	—	—	—	0.52	—
PCSSNP0546	*BRIP1*	17_59761166_C_CA	p.Ala1081fs	Cancer	55	10.46	No	3 + 3 = 6	T2c	N0	M0	2.56	No
PCSSNP0963	*CHEK2*	22_29083962_G_A	p.R519*	Cancer	51	1390	No	4 + 4 = 8	T3b	N1	M1	2.47	Yes
PCSSNP0133	*HOXB13*	17_46805906_C_A	p.Gly17Val	Noncancer	72	6.32	No	—	—	—	—	0.33	—
PCSSNP1139	*HOXB13*	17_46805561_C_T	p.Gly132Glu	Cancer	69	6.1	No	3 + 4 = 7	T2a	N0	M0	1.47	No
PCSSNP1244	*HOXB13*	17_46805561_C_T	p.Gly132Glu	Cancer	70	9.03	No	3 + 4 = 7	T1c	N0	M0	0.47	No
PCSSNP0750	*HOXB13*	17_46805561_C_T	p.Gly132Glu	Cancer	70	8.81	No	3 + 3 = 6	T1c	N0	M0	0.38	No
PCSSNP0423	*HOXB13*	17_46805561_C_T	p.Gly132Glu	Cancer	73	7.02	No	3 + 3 = 6	T2a	N0	M0	0.34	No
PCSSNP0831	*HOXB13*	17_46805561_C_T	p.Gly132Glu	Cancer	61	65.78	No	5 + 4 = 9	T3b	N0	M0	1.04	Yes
PCSSNP1305	*NBN*	8_90949282_C_A	p.Glu736*	Noncancer	72	4.08	No	—	—	—	—	0.49	—
PCSSNP0109	*NBN*	8_90949282_C_A	p.Glu736*	Cancer	77	7.6	No	3 + 3 = 6	T1c	N0	M0	1.50	No
PCSSNP0343	*PALB2*	16_23646627_G_A	p.Arg414Ter	Cancer	66	16.69	No	4 + 3 = 7	T1c	N0	M0	0.45	No

[a]= adenine; C = cytosine; G = guanine; PCa = prostate cancer; PRS = polygenic risk score; PSA = prostate-specific antigen; T = thymine.

could potentially substitute or complement a family history of PCa in choosing patients who may benefit from earlier PSA screening. The prevalence of rare variants in monogenic genes associated with PCa was approximately half that reported in European ancestry; however, these variants were independent of PRS and could provide valuable information on PCa susceptibility in Japanese. A combined assessment of both common variants and rare monogenic variants could improve PCa diagnosis.

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Data Availability

Sequence data is deposited at the Japanese Genotype-phenotype Archive (JGA, https://humandbs.biosciencedbc.jp/en/), which is hosted by the DDBJ, under accession number JGAS000487.

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